

IN THE UNITED STATES PATENT AND TRADEMARK OFFICE

Applicant : Matthew C. Coffey Art Unit : 1642
Serial No. : 09/985,756 Examiner : Lei Yao, Ph.D.
Filed : November 6, 2001
Title : METHODS FOR THE TREATMENT OF CELLULAR PROLIFERATIVE
DISORDERS

Mail Stop Amendment

Commissioner for Patents
P.O. Box 1450
Alexandria, VA 22313-1450

DECLARATION OF MATTHEW C. COFFEY, PH.D. UNDER 37 CFR § 1.131

I, Matthew C. Coffey, declare as follows:

1. I am the sole inventor of the above-identified patent application.
2. Prior to April, 2000, the invention claimed in the currently pending claims (claims 1-10) had been reduced to practice, as evidenced by pages 127, 128 and 141-142 of my doctoral dissertation (attached herewith as Exhibit 1). During my doctoral work, I conceived of the idea that neoplastic cells susceptible to reovirus infection may have constitutive MAP kinase activation. Therefore, I incubated a panel of cells in the presence or absence of serum, and determined the state of phosphorylation of the MAP kinase in these cells. As shown in Figure 6.3 (pages 141-142) of my dissertation, MDA-MB-468 cells, which are susceptible to reovirus, had phosphorylated MAP kinase regardless of the the presence of serum. In contrast, a cell that cannot be infected with reovirus (HBL-100) depended on serum to induce MAP kinase phosphorylation. These results are representative of the results obtained from a total of 22 infectable and uninfected cells derived from breast tumors, glioblastomas, pancreatic tumors and prostate tumors. Therefore, I concluded that constitutive MAP kinase activation is correlated with sensitivity to reovirus infection (last two lines on page 127 to page 128).

CERTIFICATE OF MAILING BY EXPRESS MAIL

Express Mail Label No. EV584755165US

May 9, 2005

Date of Deposit

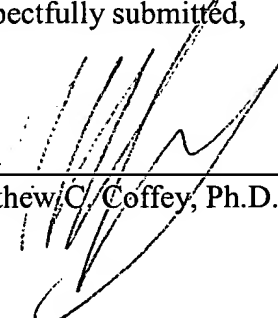
Applicant : Matthew C. Coffey
Serial No. : 09/985,756
Filed : November 6, 2001
Page : 2 of 2

Attorney's Docket No.: 16596-014001

I hereby declare that all statements made of our own knowledge are true and that all statements made on information and belief are believed to be true. I understand that willful false statements and the like are punishable by fine or imprisonment, or both (18 U.S.C. § 1001) and may jeopardize the validity of the application or any patent issuing thereon.

Respectfully submitted,

Date: May 5/05



Matthew C. Coffey, Ph.D.

Oncolytics Biotech Inc.
Suite 210
1167 Kensington Crescent, N.W.
Calgary, Alberta T2N 1X7
CANADA

**6. MEK ACTIVITY IS NOT REQUIRED FOR REOVIRUS INFECTION
ALTHOUGH ITS ACTIVITY IS A DIAGNOSTIC INDICATOR OF
REOVIRUS SUCCEPTIBILITY.**

ABSTRACT

We have previously demonstrated that when reovirus resistant NIH-3T3 cells are transformed with elements of the Ras signalling pathway, including EGFR, v-erbB, v-src, Sos, and Ras itself, there is a dramatic conferred susceptibility to reovirus infection (Strong, 1998; Coffey; 1998). To determine if it is the resultant activation of the downstream mitogen activated protein kinase (MAPK) family that is responsible for the increased susceptibility, reovirus infectable Ras transformed cells were treated with the MEK inhibitor PD98059 and then challenged with reovirus. The results indicate that at concentrations of PD98059 that effectively block ERK1/2 phosphorylation there is no block in reovirus replication. These results suggest that MEK activation does not confer susceptibility to reovirus. Although it appears that reovirus infection does not require MEK activity for infection, its activity reflects the activity of the Ras pathway. In an effort to develop an effective diagnostic indicator of reovirus infectability, we

examined a panel of 22 different human tumour cell lines to determine if constitutive ERK1/2 activation (phosphorylation) in the absence of mitogen is correlative to sensitivity to reovirus infection. Our results indicate that in 21 of the 22 samples, ERK1/2 activation was correlated with susceptibility to reovirus infection *in vitro*.

Figure 6.3. Correlation of elevated ERK1/2 phosphorylation with reovirus susceptibility of tumour cells.

(A) Immunoprecipitation of reovirus proteins with antibodies to total reovirus. Reovirus resistant HBL-100 breast tissue derived cells and reovirus susceptible MDA-MB-468 breast tumour cells were infected or mock-infected for 48 hours and then pulse labeled with [35S] methionine for 2 h. Cells were then harvested and prepared lysates were subjected to immunoprecipitation. Reovirus proteins are indicated on the right. (B) Level of ERK1/2 phosphorylation in the absence or presence of serum. HBL-100 or MDA-MB-468 cells were grown in 10% FBS or were serum starved for 48 h. Cells were washed and then lysed in protein sample buffer (PSB). Lysates were then subjected to SDS-PAGE followed by western blotting with antibodies to the phosphorylated form of ERK1/2. (C) Total ERK1/2 levels in these cells. These results are representative of results obtained from infectable and uninfected cells derived from breast tumours, glioblastomas, pancreatic tumours and prostate tumours.

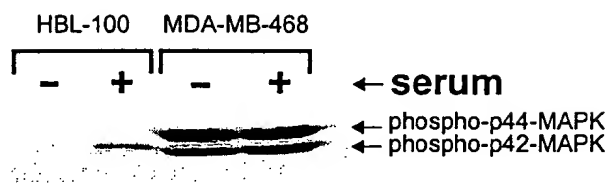
A

Immunoprecipitation



B

Phospho-MAPK



C

Total MAPK

